## Osteoinductive and Antibacterial Biomaterials for Bone Tissue Engineering

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2016

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Publisher's PDF, also known as Version of record

# Chapter 7

# General Summary

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Nowadays, many patients with missing teeth or bone defects have gained satisfactory outcomes from the development of dental implants and bone substitutes. However, for patients with well-recognized risk factors, such as diabetes, metabolic bone disorders, or with bone defects that are too large to self-heal, or with locally infected bone defects, we are in significant need to improve the properties of dental implants and bone substitutes to achieve more satisfactory osseointegration and bone regeneration.

To some extent, osseointegration of dental implants and bone regeneration caused by bone substitutes share the same essence, which is forming new bone along the material─either dental implants or bone substitutes. Based on what we understand about the three key factors (Osteogenic cells, scaffolds, growth factors) in bone tissue engineering, dental implants and bone substitutes can function as scaffolds in the process of bone formation, we believe that it can be an effective strategy to introduce growth factors to these scaffolds in order to improve bone formation─either osseointegration or bone formation.

Based on this theory, our group has developed various osteoinductive materials by introducing a well-known and effective growth factor─BMP2 to many kinds of calcium phosphate materials. Firstly, we developed a BMP2-incorporated biomimetic calcium phosphate coating which can be applied to functionalize biomaterials, such as dental implants. The biomaterial is first immersed in a 5-fold-concentrated simulated body fluid for 24 hours at 37°C to gain a fine, dense layer of amorphous calcium phosphate on it. Serving as a seeding substratum for the following deposition, the biomaterial with the first amorphous layer is then immersed in a supersaturated calcium phosphate solution with BMP2 for 48 hours at 37°C to gain a more substantial crystalline layer with incorporated BMP2. This biomimetic coating was proven by the success in introducing osteoinductivity to a broad range of biomaterials, such as metallic, inorganic, polymeric materials that have completely different geometries, topographies and surface chemistries. Albeit so, this type of biomimetic coating on bone-defect-filling materials has the limitation that its growth relies highly on the proper surface roughness and/or active surface chemistry of the bone-defect-filling materials. We therefore modified the biomimetic coating technique and developed a novel granule to break through these limitations. The process of preparing the two-layer coating is repeated three times to obtain a novel 3-dimensional layer-by-layer assembled BMP2-coprecipitated biomimetic calcium phosphate granules (BMP2-cop.BioCaP). As an osteoinducer, it functions perfectly to introduce osteoinductivity to osteoconductive bone substitutes, simply by being mixed with them, thus enhancing bone regeneration. However, it cannot be used as an independent bone-defect-filling material because it degrads so fast that it cannot function as scaffold for osteogenic cells to attach

and form new bone. For this reason, we modified the protocol for preparation of BMP2-cop.BioCaP and successfully developed a novel BMP2-incorporated biomimetic calcium phosphate (BMP2-BioCaP). Compared to BMP2-cop.BioCaP, it showed a slower degradation rate which matched better with the ingrowth of bone tissue in the process of bone regeneration.

This thesis is built on the various applications of these three generations of biomimetic materials. Firstly, we've already conducted intensive research on the BMP2-incorporated biomimetic calcium phosphate coating in previous studies. In this thesis, besides this coating, we also reviewed many other coatings to enhance and accelerate the osseointegration of metallic implants. Secondly, we examined the function of BMP2-cop.BioCaP as an osteoindcuer in a preclinical study. We combined BMP2-cop.BioCaP with clinically often used osteoconductive bone substitute─biphasic calcium phosphate (BCP) in order to introduce osteoinducivity to the bone substitutes and evaluate if it can improve bone regeneration in critical-sized bone defects (CSBD). Thirdly, we modified the BMP2-BioCaP as not only an osteoinductive bone substitute, but also an antibiotic carrier. The hypothesis was that it can function as an osteoinductive and antibacterial biomaterial system for treatment of infected critical-sized bone defects. Both *in-vitro* and *in-vivo* experiments are performed to evaluate its osteoinductivity and antibacterial activity.

The aims described in the first chapter are addressed as follows:

1. *To introduce osteoinductivity to clinically used BCP by a novel osteoinducer and evaluate if it can improve bone regeneration in CSBD. Furthermore, to explore the mechanism of the osteoinducer enhancing bone regeneration.*

BCP is a clinically often used bone-defect-filling material with excellent osteoconductivity. However, it may be not enough for some cases in clinical practice, such as patients associated with diabetes, local osteoporosis and metabolic bone disorder which can compromise bone healing. It is often mixed with autologous bone to gain osteoinductivity for advanced bone regeneration. Due to the limitations of applying autologous bone, we previously developed the BMP2-cop.BioCaP in aim to replace it, which can slowly release BMP2 at a steady rate from the 3$^{rd}$ day until 35$^{th}$ day. In **chapter 2**, when repairing a CSBD model─8mm rat cranial defect, BCP mixed with BMP2-cop.BioCaP or autologous bone showed a comparable amount of newly formed bone at both 4 and 12 weeks, which was significantly more than BCP alone. This confirmed that BMP2-cop.BioCaP, a potential osteoinducer, can function as effectively as autologous bone to improve bone regeneration in CSBD.

To better understand the mechanism of BMP2-cop.BioCaP improving bone regeneration, we need to get deeper insights into the results. In group of BCP alone, the new bone was

only found on the periphery of the bone defect in contact with the host bone, which was named as osteoconductive bone. Whereas, in groups of BCP combined with BMP2-cop.BioCaP or autologous bone, newly formed bone was found not only osteoconductive bone, but also osteoinductive bone─the new bone in the center of the bone defect without any contact with the host bone. What's more, osteoinductive bone grew in time till 12 weeks as observed in this study, but osteoconductive bone stopped growing after 4 weeks. Therefore, we believe that the mechanism of BMP2-cop.BioCaP improving bone regeneration in CSBD was that (1) besides the bone-growing center along host bone lining the defect, it brought a new bone-growing center in the middle of defect; (2) it prolonged grow profile of newly formed bone thanks to the spontaneous growth of osteoinductive bone.

2. *To develop an antibacterial and osteoinductive biomaterial for treatment of infected CSBD and to evaluate its antibacterial activity and osteoinductivity in vitro and in vivo.*

Treatment of infected critical-sized bone defects remains a great challenge in orthopedic and oral and maxillofacial surgery because of the residual bacteria that is impossible to completely eliminate by debridement and beyond-self-healing bone defects. To overcome these two difficulties and consequently repair the infected critical-sized bone defects, we believe the ideal local biomaterial system should meet the following requirements: 1) The biomaterial system can function as both antibiotic carrier and bone substitute, to not only clear the infection but to also contribute to the subsequent bone regeneration process; 2) The antibiotics used for local delivery should have a broad spectrum of activity and a low rate of bacteria resistance; 3) The antibiotic should also be delivered to its optimal concentration, at which it reaches the balance between cellular toxicity and antibacterial activity; 4) Osteoinductive bone grafts are more favorable for improving bone regeneration; 5) The release kinetics of antibiotic and osteoinductive agents should meet their optimal delivery mode respectively.

In **chapter 3**, we used the osteoinductive BMP2-BioCaP as the carrier of a powerful antibacterial agent─hydroxypropyltrimethylammonium chloride chitosan (HACC) to fabricate a BMP2-BioCaP/HACC complex. HACC showed strong antibacterial acitivity to MRSA. Although many antibiotics are naturally associated with the issue of their cytotoxicity, we could still determine the most balanced concentration of HACC─40μg/mL, at which HACC can kill bacterial without harming pre-osteoblasts. At this concentration, HACC also did not show negative influence on proliferation and BMP2-induced differentiation of pre-osteoeblasts. The corresponding amount of HACC was therefore loaded on the BMP2-BioCaP to fabricate an antibacterial and non-cytotoxic BMP2-BioCaP/HACC complex. The release kinetic of this complex confirmed it as a sequential release system: burst release of HACC and followed by controlled release of BMP2. Given

that long-term usage of low-dosage antibiotics is highly associated with bacterial resistance, we logically speculate that burst release of a powerful antibiotic to kill possible site-infection related bacteria. What's more, it is consensus that slow release BMP2 at a relatively low concentration is best for its effectiveness. Therefore, it delivered both HACC and BMP2 at their optimal delivery mode respectively, which guaranteed its antibacterial activity and osteoinducvitity *in vivo*. We observed that new bone formation was induced by the BMP2-BioCaP/HACC complex in a model of subcutaneous sites in rats.

3. *To review the biological process of osseointegration and offer an overview of the coatings designed for improving osseointegration of metallic biomaterials*

Like introducing BMP2 into bone substitute materials is an efficient and effective way for mateirals to gain osteoinductivity, osseointegration of metallic biomateirals can also be improved by incorporating BMP2 into metallic biomaterials coating. In **chapter 4**, we firstly reviewed the biological process of osseointegration at molecular level. The first step of the body's reaction to a biomaterial is the adsorption of the molecules onto the surface of biomaterials from the surrounding fluid, which is followed by attachment and proliferation of mesenchymal stem cells. Thanks to various growth factors, the multipotent mesenchymal stem cells can differentiate into osteoblasts. Thirdly, osteoblasts produce cross-linked collagen to form the organic matrix of bone, which is then calcified to form a mineralized bone matrix. The fourth stage is the remodeling phase, in which the woven bone is transformed into lamellar bone and osseointegration of metallic implants is finally established. Various kinds of coatings on implant surfaces have been designed to regulate these four phases to promote osseointegration, such as extracellular matrix for improving recognition and adhesion of cells, BMP2 for enhancing proliferation and differentiation of pre-osteoblasts fluoride for promoting mineralization and matrix, bisphosphonates for regulating bone remodeling and so on. However, most of them are on the stage of preclinical studies, only hydroxyapatite and bisphosphonate coatings have been evaluated in clinical trials.

4. *To evaluate the accuracy of measuring bone thickness surrounding dental implants and the reliability of assessing existence and completion of osseous integration of augmentation material using a Cone beam computered tomography (CBCT).*

In clinical practice, CBCT is widely used for pre-operative assessment of bone quantity and quality in the region of interest, and for post-operative evaluation of bone regeneration after sinus lift augmentation and osseointegration of dental implants. In **chapter 5**, the accuracy of CBCT was evaluated by comparing the same measurements with golden standard─histological images. The results indicated that this CBCT system allows reliable measurements of peri-implant bone thickness at an accuracy of half a millimeter and

assessing the existence and integration of bone augmentation materials. However, it is not possible to evaluate whether the implant is covered completely by hard tissue.